

# Sucrose metabolism: *Anabaena* sucrose-phosphate synthase and sucrose-phosphate phosphatase define minimal functional domains shuffled during evolution

Andrea Cumino<sup>1</sup>, Leonardo Curatti<sup>1</sup>, Laura Giarrocco, Graciela L. Salerno\*

Centro de Investigaciones Biológicas, FIBA, Mar del Plata, Argentina

Received 27 December 2001; revised 5 February 2002; accepted 6 February 2002

First published online 8 March 2002

Edited by Robert B. Russell

**Abstract** Based on the functional characterization of sucrose biosynthesis related proteins [SBP: sucrose-phosphate synthase (SPS), sucrose-phosphate phosphatase (SPP), and sucrose synthase (SuS)] in *Anabaena* sp. PCC7120 and sequence analysis, we have shown that SBP are restricted to cyanobacterium species and plants, and that they are multidomain proteins with modular architecture. *Anabaena* SPS, a minimal catalytic SPS unit, defines a glucosyltransferase domain present in all SPSs and SuSs. Similarly, *Anabaena* SPP defines a phosphohydrolase domain characteristic of all SPPs and some SPSs. Phylogenetic analysis points towards the evolution of modern cyanobacterial and plant SBP from a bidomainal common ancestral SPS-like gene. © 2002 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

**Key words:** Cyanobacterium; Sucrose-phosphate synthase; Sucrose-phosphate phosphatase; Sucrose synthase; Modular arrangement; Phylogenetic analysis

## 1. Introduction

Sucrose is one of the most abundant products in nature. Most photosynthetic eukaryotes and some species of oxygenic photosynthetic prokaryotes synthesize sucrose [1,2]. In higher plants, sucrose occupies a unique position, comparable only to glucose in the animal world [3]. It is a major product of photosynthesis, with a central role as a transport sugar, in growth, development, storage, signal transduction and stress [1,3–5]. In cyanobacteria and chlorophyta, although it is associated with environmental stress responses [2,6–8], its function has not been totally elucidated.

Sucrose-phosphate synthase (SPS, UDP-glucose:D-fructose-

6-phosphate 2- $\alpha$ -D-glucosyltransferase, EC 2.4.1.14), sucrose-phosphate phosphatase (SPP, sucrose-6<sup>F</sup>-phosphate-phosphohydrolase, EC 3.1.3.24) [1,8–12], and sucrose synthase (SuS, UDP-glucose:D-fructose 2- $\alpha$ -D-glucosyltransferase, EC 2.4.1.13) [9,10], which we named sucrose biosynthesis-related proteins (SBP), have been well characterized in plants and unicellular eukaryotes. Much less is known about sucrose metabolism in prokaryotes. The biosynthesis of sucrose through the action of SPS and SPP has been recently identified in the cyanobacterium *Anabaena* sp. PCC7119 and *Synechocystis* sp. PCC6803 [13,14]. The occurrence of SuS has only been reported in *Anabaena* sp. and in other filamentous nitrogen-fixing cyanobacteria [15,16]. To date only the identification and characterization of the genes encoding *Synechocystis* SPS (*spsA*), and *Anabaena* SuS (*susA*) and SPP (*sppA*) have been reported [14,16,17].

The present study shows that *Anabaena* SPS, minimal catalytic SPS unit, defines a glucosyltransferase domain (GTD) present in all SPSs and SuSs, and that SBPs are domainal proteins that may have originated from a common ancestral SPS-like gene within the cyanobacterial phylogenetic radiation.

## 2. Materials and methods

### 2.1. Bacterial strains and growth

*Anabaena* sp. strain PCC7120 was grown in BG-11 medium as described [13]. *Escherichia coli* DH5 $\alpha$  and BL21(DE3):pLysS (Novagen) strains were grown in Luria-Bertani medium supplemented with 50  $\mu$ g/ml carbenicillin.

### 2.2. Cloning and expression of SPS genes

*Anabaena* genomic DNA purification and PCR were carried out as described [16]. Plasmids were isolated and modified according to standard protocols [18]. Two DNA sequences (ORF154 and ORF287) homologous to Sy-SPS were obtained by BLAST searches [19] against the *Anabaena* sp. PCC7120 genome (Kazusa DNA Research Institute, <http://www.kazusa.or.jp>). PCR methodology was used to amplify the open reading frames (ORFs) or genomic regions, and the products were ligated to pRSET-A (Invitrogen) or to pGEM-T Easy (Promega), respectively. The resulting constructs were introduced into *E. coli* strain BL21( $\lambda$ DE3):pLysS (Novagen) or DH5 $\alpha$  to produce His<sub>6</sub>-tagged or non-tagged recombinant proteins, respectively.

### 2.3. Protein purification and enzyme assays

His<sub>6</sub>-tagged An-SPS-A and An-SPS-B fusion proteins were purified by Co<sup>2+</sup> affinity chromatography (TALON resin, Clontech). An-SPSs were also expressed from *Anabaena* genomic regions in *E. coli* to produce non-tagged recombinant SPSs, which were purified by DEAE-Sepharose chromatography [13]. Extracts from *Anabaena* cells, further purification of An-SPSs, and enzyme activity were performed as described [13].

\*Corresponding author. Fax: (54)-223-475 7120.

E-mail address: fibamdq@infovia.com.ar (G.L. Salerno).

<sup>1</sup> These authors contributed equally to this work.

**Abbreviations:** GTD, glucosyltransferase domain; PHD, phosphohydrolase domain; SBP, sucrose biosynthesis-related proteins; SPP, sucrose-phosphate phosphatase; SPS, sucrose-phosphate synthase; SuS, sucrose synthase; The prefixes indicate: An-, *Anabaena* sp. PCC7120; An7119-, *Anabaena* sp. PCC7119; At-, *Arabidopsis thaliana*; Mt-, *Medicago truncatula*; Os-, *Oryza sativa*; Np-, *Nostoc punctiforme*; PmMED-, *Prochlorococcus marinus* MED4; PmMIT-, *P. marinus* MIT9313; St-, *Solanum tuberosum*; Sm-, *Synechococcus marinus* WH8102; Sy-, *Synechocystis* sp. PCC6803; Zm-, *Zea mays*

#### 2.4. Antibody preparation and immunoassays

Anti-An-SPS-A was prepared in rabbits using the His<sub>6</sub>-tagged recombinant protein [20]. Western blots and immunotitration of SPS activity were performed as described [17].

#### 2.5. Sequence analysis

The sequences reported in this paper have been deposited in the EMBL database (accession numbers AJ302071, AJ302072, and AJ316584–AJ316596). Other sequences were obtained from the non-redundant protein databases of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>), the Kazusa DNA Research Institute, and the Department of Energy Joint Genome Institute (<http://www.jgi.doe.gov>) by BLAST searches [19]. ORFs were scored as SBP homologues for *E*-values of  $\leq 10^{-25}$  when compared with genes of established biochemical function [An7119-SuS-A (AJ010639), Sy-SPS (*sr*0045) and An-SPP (AJ302073)]. Other cyanobacterial and plant sequences used are: An-SuS-A (AJ316595 and AJ316596), An-SuS-B (AJ316584), An-SPS-A (AJ302071), An-SPS-B (AJ302072), Np-SuS-A (AJ316590), Np-SuS-B (AJ316590), Np-SPS-A (AJ316587), Np-SPS-B (AJ316594), Np-SPS-C (AJ316588), Np-SPP-A (AJ316585), Np-SPP-B (AJ316586), PmMED-SPS (AJ316591), PmMIT-SPS (AJ316592), Sm-SPS (AJ316594), Sy-SPS (*sr*0953), and At-SuS-1 (AB0016872), At-SuS-2 (AB17068), At-SuS-3 (AL353871), At-SuS-4 (AF075597), At-SuS-5 (AC012396), At-SPS-1 (AL049487), At-SPS-2 (AL391222), At-SPS-3 (AC004809), At-SPP-1 (AL132972), At-SPP-2 (AC0224261), At-SPP-3 (AL132957), At-SPP-4 (AC007017), Mt-SPP (AF283566), Os-SuS-1 (X64770), Os-SPS (T04103), St-SuS (U24087), St-SPS (Q43845), Zm-SuS-1 (X02400), ZmSPS (P31927), Zm-SPP (AF283564). Sequence alignments were generated with the CLUSTALX software program (version 1.8) [21] and dendrograms were compiled using the neighbor-joining method (computed from 1000 independent trials) of CLUSTALX and the maximum parsimony algorithm of the PHYLIP package [22].

### 3. Results

#### 3.1. Functional identification of SBP in *Anabaena* sp.

BLAST searches using as query Sy-SPS revealed five homologous ORFs in the *Anabaena* sp. PCC7120 genome. Interestingly, the highest scores corresponded to two ORFs 99% identical to An7119-SuS-A and SuS-B ([16] and unpublished work). Another ORF has been recently identified as encoding An-SPP [17]. The remaining ORF154 and ORF287, which encode proteins of 47 189 and 46 765 kDa respectively, were identified in this study as functional SPS genes (*sp*sA and *sp*sB) as they conferred SPS activity to *E. coli*. Recombinant proteins and the enzymes purified from *Anabaena* show similar substrate specificity, pH dependence, kinetic parameters (not shown), elution profile in DEAE-Sephacel chromatography, immunoreactivity, and polypeptide relative molecular mass (Fig. 1). Thus, An-SPS-A and An-SPS-B encoded by *sp*sA and *sp*sB are orthologous proteins with the formerly described An7119-SPS-I and An7119-SPS-II, respectively [13].

#### 3.2. Domainal nature of SBP

BLAST searches using as query Sy-SPS, An-SPS-A, An7119-SuS-A and An-SPP, and multiple sequence alignments (Fig. 1, supplementary material, <http://www.elsevier.com/PII/S0014579302025164>) showed that SBP are restricted to cyanobacteria and plants. All the microbial SBP sequences available in public databases were included in the alignments, whereas only some representative plant sequences were used as they show a high amino acid identity (> 51%) among each enzyme group [12,14,16]. All SPSs and SuSs share considerable homology (20–33%) in a 400 amino acid region (Fig. 2 and Fig. 1, supplementary material). This region exactly matches An-SPSs, the smallest proteins with SPS activity (Fig. 1A), defining a functional glucosyltransferase domain

(GTD) that contains a conserved motif exclusive of SPSs and SuSs and another motif that is ubiquitous in members of the NRD1 $\alpha$  glycosyltransferase family (Figs. 2 and 3, boxes I and II, respectively and Fig. 1, supplementary material) [23]. However, the substrate discrimination between SPSs and SuSs based on the presence of a K or V residue in the former motif [24] must be discarded (Fig. 3). On the other hand, the two highly conserved E residues in the latter motif have been proposed to be involved in the binding of UDP-Glc (Fig. 3, box II) [23]. The UDP-Glc binding site proposed for plant SPSs (22 amino acids adjacent to box I towards the C-terminal end in Sy-SPS) [25] is not conserved in plant SuSs or in cyanobacterial SBP (Fig. 1, supplementary material). Consequently, we propose the following signature for SPS and SuS proteins: [DE]-X-G-G-Q-X(2)-Y-[VIL]-X-[DE]-X(300, 430)-E-X-F-G-X(3)-E-X(6)-P-X(2)-A-[TS]-X-G-G (Fig. 3). Additionally, some SPSs and all SPPs share a 250 amino acid region (Fig. 2 and Fig. 1, supplementary material) that exactly matches An-SPP, the minimal protein with SPP activity [17], defining a phosphohydrolase domain (PHD) of the SBP. Conserved motifs among phosphohydrolases [26] and the L2-haloacid dehalogenase superfamily [27] are found in all SPPs, and in PmMIT-SPS and Sm-SPS (Figs. 2 and 3, boxes III and IV and Fig. 1, supplementary material). Other structural features of SBP are the N-terminal extension of 350 amino acids at the GTD of all SuSs, the 178 amino acid N-terminal extension at the GTD of plant SPSs, and the 150 amino acid C-terminal extension at the PHD of plant SPPs (Fig. 2). Taken together, these results strongly suggest that SBPs are multi-domain proteins that may share structural features.

#### 3.3. An SPS-like gene gave origin to SBP

SBP have been found in all cyanobacterial strains examined. A coincidental and unique SBP was identified in the

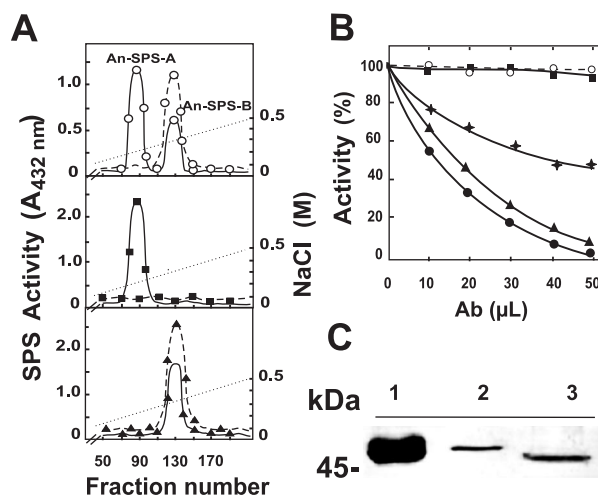


Fig. 1. Identification of two SPS genes in *Anabaena* sp. PCC 7120. A: DEAE-Sephacel chromatography of *Anabaena* (○), and *E. coli* expressing An-SPS-A (■) or An-SPS-B (▲) extracts. Enzyme activity was measured using ADP-Glc (dotted line) or UDP-Glc (solid line). B: Neutralization of SPS activity by anti-An-SPS-A. Purified An-SPS-A (▲); His<sub>6</sub>-tagged An-SPS-A (●); His<sub>6</sub>-tagged An-SPS-B (◆). The corresponding 100% activities were 0.8, 40 and 11 nkat/mg protein, respectively. Dotted line, control incubation of An-SPS-A with pre-immune serum (○). C: Immunoblot analysis using anti-An-SPS-A. Lane 1, His<sub>6</sub>-tagged An-SPS-A (2 μg); lane 2, His<sub>6</sub>-tagged An-SPS-B (3 μg); lane 3, SPS-A purified from *Anabaena* cells (0.5 μg). Proteins were separated on 15% SDS-PAGE.

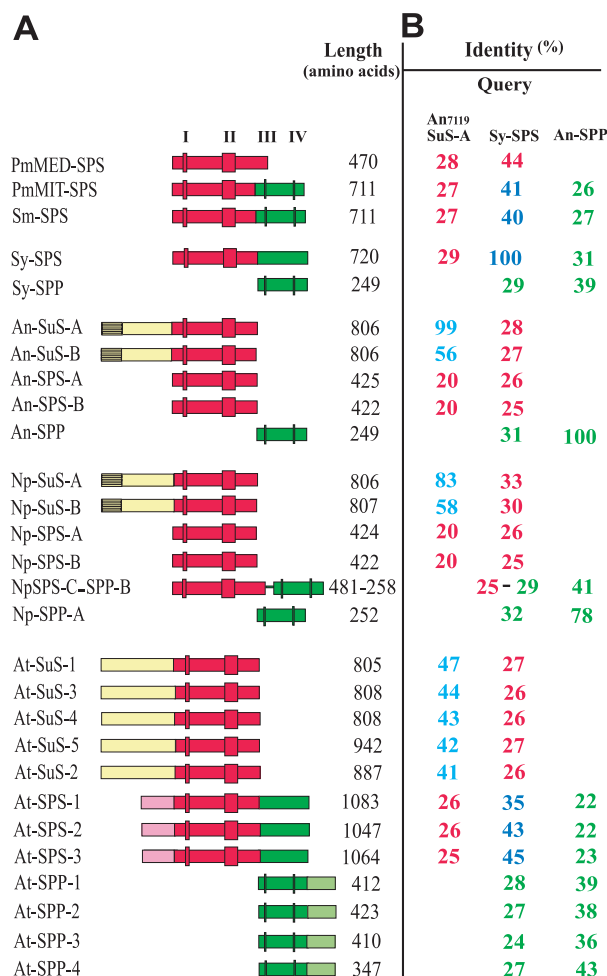


Fig. 2. Schematic representation of the organization of SBP. A: Sequence relationships were deduced after BLASTp and CLUSTALX analysis. GTD, red box; PHD, green box; characteristic N-terminal SuS extension, yellow box (hatched region represents the residues not conserved between cyanobacterial and plant SuSs); N-terminal plant SPS extension, pink box; C-terminal plant SPP extension, light green box. B: Percentage amino acid identity between An7119-SuS-A, Sy-SPS or An-SPP, and retrieved SBP sequences after BLASTp analysis. Red numbers indicate that the % identity corresponds only to the GTD and green numbers only to the PHD. When Sy-SPS (which contains both domains) was used as query, % identity is colored blue indicating that the aligned region corresponds to both domains. When An-SuS-A was used as query, % identity is colored light blue indicating that the aligned region corresponds to the GTD and the specific SuS N-terminal extension. I–IV, proposed fructose 6-phosphate/fructose binding site, NRD1α glycosyltransferase family motif, and phosphohydrolase superfamily motifs, respectively.

picoplanktonic open ocean cyanobacteria *Prochlorococcus marinus* MED4, *P. marinus* MIT9313 and *Synechococcus marinus* independently of the query sequence used (Fig. 2). In *P. marinus* MIT9313 and *S. marinus*, the GTDs share about 27 and 41% identity with An7119-SuS-A and Sy-SPS respectively, while the PHDs are about 27% identical to An-SPP. GTDs and PHDs of the open ocean cyanobacteria cluster together with those of plant and other cyanobacterial SPSs in a highly bootstrap-supported neighbor-joining tree (Fig. 4). Qualitatively similar tree topologies were observed when using a maximum parsimony algorithm (not shown). In *Synechocystis* sp. PCC6803, in addition to the bidomain Sy-SPS, there is

an autonomous PHD (Sy-SPP) that clusters together with functionally characterized SPPs (Fig. 4B) [12,17]. The PHDs of SPSs and SPPs form two closely related clusters (Fig. 4B). In *Anabaena* sp. and *Nostoc* sp. there are five and seven SBPs, respectively. Np-SPS-C and Np-SPP-B are adjacent and separated by a 165 bp spacer. Notably, SuS-A homologues are only present in those filamentous cyanobacteria and in plants. The GTDs of SuSs form a closely related sister clade with the GTDs of SPSs (Fig. 4A). The occurrence of specific clusters for each plant SBP indicates that they originated by recent gene duplications of their respective single ancestors within the plant lineage (Fig. 4).

#### 4. Discussion

Biochemical and sequence analysis strongly suggest that SBP are domainal proteins [28], sharing primordial functional domains (GTD and PHD). SPSs support SBP modularity since three different domainal arrangements can be described: the minimal SPS unit (GTD) like An-SPSs, the bidomain SPS prototype (GTD-PHD), like Sy-SPS, and plant SPSs (N-terminal extension-GTD-PHD) with an additional regulatory region (Fig. 2) [9]. A comparable domainal organization was found in the proteins responsible for the biosynthesis of tre-

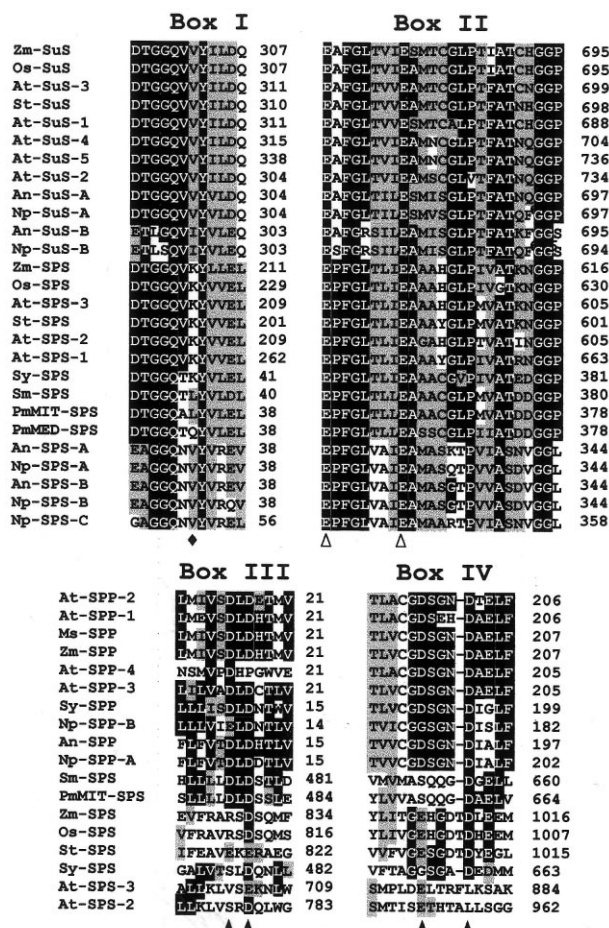


Fig. 3. Multiple sequence alignments of highly conserved motifs in GTD (boxes I and II) and PHD (boxes III and IV). Identical residues are black; conservative amino acid substitutions are gray shaded; V/K residue proposed to discriminate the binding of Fru/Fru-6P (◆); conserved E residues (Δ); conserved D residues (▲).



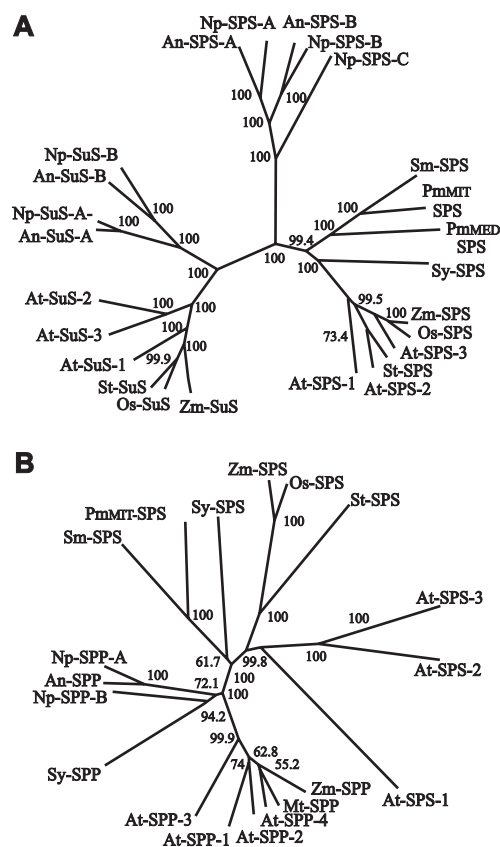


Fig. 4. Phylogenetic analysis of SBP. Unrooted neighbor-joining phylograms were constructed after sequence alignment of (A) GTD or (B) PHD, with the CLUSTALX program using a BLOSSUM matrix and a bootstrap trial of 1000. The graphical representations of the trees were generated using DRAWTREE of the PHYLIP package.

halose [29]. It is likely that GTD and PHD extensions of plant SPSs and SPPs and of all SuSs may be involved in oligomerization, since only cyanobacterial SPSs and SPPs are monomeric proteins [13,15,17].

The analysis of the occurrence of SBP in complete genomes was useful to shed light on the origin and evolution of the sucrose metabolic pathway. The presence of bidomain SPSs in open ocean species, phylogenetically located at the base of the cyanobacterial radiation [30,31], led us to suggest that sucrose metabolism originated about 2–3 billion years ago [32] from an ancestral SPS-like gene (Fig. 2, supplementary material, <http://www.elsevier.com/PII/S0014579302025164>). The first glycosyl acceptor may have been fructose 6-phosphate (and not fructose) because of its abundance in the intermediate metabolism. Moreover, the hydrolysis of the intermediate sucrose 6-phosphate ( $\Delta G^\circ = -16.5$  kJ/mol) [33] leads to an essentially irreversible sucrose biosynthesis pathway. In *Synechocystis*, which diverged from the main cyanobacterial lineage more recently than the open ocean strains [34], SPP appears to have arisen after PHD duplication from a common ancestral SPS-like gene. Sequence erosion at the PHD of SPSs is more evident in those species that have autonomous SPPs (Figs. 3 and 4B), suggesting that PHDs of those SPSs are functionally redundant. The outcome of autonomous SPPs may have provided sucrose biosynthesis with a new level of regulation based on protein–protein interaction and the chan-

nelling of sucrose 6-phosphate, as has been proposed for plant SPS/SPP [35]. A later gene duplication of the GTD from a SPS-like gene gave rise to SuS, which identity was completed by the addition of an N-terminal extension. These events took place before the branching of filamentous heterocystous cyanobacteria (like *Nostoc* and *Anabaena* sp.), that arose significantly after the appearance of other cyanobacterial lines and the common ancestor of chloroplasts [34,36]. The *Anabaena* SPS prototype appears to be arisen as a result of the loss of the PHD in the heterocystous cyanobacterium lineage. The special arrangement of Np-SPS-C and Np-SPP-B (Fig. 3), closely related to the Sy-SPS and Sy-SPP (Fig. 4), may support the vertical transfer of a bidomain SPS-like through evolution and suggest that the GTD-PHD split might have happened several times, including the case of PmmED-SPS.

The cyanobacterial endosymbiotic origin of plant chloroplasts is generally accepted [36]. Most of the endosymbiotic genes were transferred to the nucleus, but their products have been preferentially reimported to the organelle, where they do not interfere with the host cytoplasmic metabolism [37]. Nevertheless, chloroplasts did not retain the sucrose biochemistry of the free-living ancestor and instead of reimporting the gene products, they gave rise to a novel cytoplasmic pathway in the plant lineage.

**Acknowledgements:** We thank M. Fernández and C. Fernández for excellent technical assistance, Prof. H.G. Pontis for helpful discussions, and E. Berry and D. Cobessi for critical reading of the manuscript. This research was supported by grants of the SEPCyT, UNMDP and FIBA. This work is part of the PhD thesis of A.C. and L.C., UNMDP, Argentina.

## References

- [1] Avigad, G. (1982) in: *Plant Carbohydrates I* (Loewus, F.A. and Tanner, W., Eds.), pp. 217–346, Springer-Verlag, Heidelberg.
- [2] Reed, R.H., Richardson, D.L., Warr, S.L. and Stewart, W.D.P. (1984) *J. Gen. Microbiol.* 130, 5–25.
- [3] Pontis, H.G. (1977) in: *Plant Biochemistry II* (Northcote, D.H., Ed.), pp. 79–117, University Park Press, Baltimore, MD.
- [4] Levitt, J. (1980) in: *Responses of Plants and Environmental Stresses* (Levitt, J., Ed.), pp. 163–179, Academic Press, New York.
- [5] Koch, K.E. (1996) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47, 509–540.
- [6] Hagemann, M. and Erdmann, N. (1997) in: *Cyanobacterial Nitrogen Metabolism and Environmental Biotechnology* (Rai, A.K., Ed.), pp. 156–220, Narosa Publishing House, New Delhi.
- [7] Potts, M. (2000) in: *The Ecology of Cyanobacteria* (Whitton, B.A. and Potts, M., Eds.), pp. 465–504, Kluwer Academic Publishers, Dordrecht.
- [8] Salerno, G.L. and Pontis, H.G. (1989) *Plant Physiol.* 89, 648–651.
- [9] Huber, S.C. and Huber, J.L. (1996) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47, 431–444.
- [10] Winter, H. and Huber, S.C. (2000) *Crit. Rev. Plant Sci.* 19, 31–67.
- [11] Salerno, G.L., Porchia, A.C. and Sánchez, N. (1995) in: *Sucrose Metabolism, Biochemistry, Physiology and Molecular Biology*, Vol. 14 (Pontis, H.G., Salerno, G.L. and Echeverria, E.J., Eds.), pp. 34–39, American Society of Plant Physiologists, Rockville, MD.
- [12] Lunn, J.E., Ashton, A.R., Hatch, M.D. and Heldt, H.W. (2000) *Proc. Natl. Acad. Sci. USA* 97, 12914–12919.
- [13] Porchia, A.C. and Salerno, G.L. (1996) *Proc. Natl. Acad. Sci. USA* 93, 13600–13604.
- [14] Curatti, L., Folco, E., Desplats, P., Abratti, G., Limones, V., Herrera-Estrella, L. and Salerno, G.L. (1998) *J. Bacteriol.* 180, 6776–6779.

- [15] Porchia, A.C., Curatti, L. and Salerno, G.L. (1999) *Planta* 210, 34–40.
- [16] Curatti, L., Porchia, A.C., Herrera-Estrella, L. and Salerno, G.L. (2000) *Planta* 211, 727–735.
- [17] Cumino, A.C., Ekeroth, C.N. and Salerno, G.L. (2001) *Planta* 214, 250–256.
- [18] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn., Cold Spring Harbor Laboratory Press, Plainview, NY.
- [19] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) *J. Mol. Biol.* 215, 403–410.
- [20] Salerno, G.L., Pagnussat, G.C. and Pontis, H.G. (1998) *Cell. Mol. Biol.* 44, 407–416.
- [21] Thomson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997) *Nucleic Acids Res.* 24, 4876–4882.
- [22] Felsenstein, J. (1993) *PHYLP (Phylogeny Inference Package)*, Version 3.5c, University of Washington, Seattle, WA.
- [23] Kapitonov, D. and Yu, R.K. (1999) *Glycobiology* 9, 961–978.
- [24] Salvucci, M.E., van de Loo, F.J. and Klein, R.R. (1995) in: *Sucrose Metabolism, Biochemistry, Physiology and Molecular Biology*, vol. 14 (Pontis, H.G., Salerno, G.L. and Echeverria, E.J., Eds.), pp. 25–33, American Society of Plant Physiologists, Rockville, MD.
- [25] Salvucci, M.E. and Klein, R.R. (1993) *Plant Physiol.* 102, 529–536.
- [26] Thaller, M.C., Schippa, S. and Rossolini, G.M. (1998) *Protein Sci.* 7, 1647–1652.
- [27] Aravind, L., Galperin, M.Y. and Koonin, E.V. (1998) *Trends Biochem. Sci.* 23, 127–129.
- [28] Doolittle, R.F. (1995) *Annu. Rev. Biochem.* 64, 287–314.
- [29] Goddijn, O.J.M. and van Dun, K. (1999) *Trends Plant Sci.* 4, 315–319.
- [30] Palenik, B. and Haselkorn, R. (1992) *Nature* 355, 265–267.
- [31] Urbach, E., Robertson, D.L. and Chisholm, S.W. (1992) *Nature* 355, 267–270.
- [32] Schopf, J.W. (2000) in: *The Ecology of Cyanobacteria* (Whitton, B.A. and Potts, M., Eds.), pp. 13–35, Kluwer Academic Publishers, Dordrecht.
- [33] Dennis, D.T. and Blakeley, S.D. (2000) in: *Biochemistry and Molecular Biology of Plants* (Buchanan, B.B., Guissem, W. and Jones R.L., Eds.), pp. 630–675, American Society of Plant Physiologists, Rockville, MD.
- [34] Giovannoni, S.J., Turner, S., Olsen, G.J., Barns, S., Lane, D.J. and Pace, N.R. (1988) *J. Bacteriol.* 170, 3584–3592.
- [35] Echeverria, E., Salvucci, M.E., González, P., Paris, G. and Salerno, G. (1997) *Plant Physiol.* 115, 223–227.
- [36] Douglas, S.E. (1994) in: *Molecular Biology of Cyanobacteria* (Bryant, D.A., Ed.), pp. 91–118, Kluwer Academic Publishers, Dordrecht.
- [37] Martin, W. and Herrmann, R. (1998) *Plant Physiol.* 118, 9–17.